REMARKS/ARGUMENTS

Claims 1, 4-11, 13-14, 17-24, and 38-40 are pending.

Claim 40 has been added.

Claims 1 and 4-11 have been withdrawn.

Claims 2-3, 12, 15-16, and 25-37 have been cancelled.

Support for the amendments is found in the claims and specification (e.g., page 3, ln. 1-5), as originally filed. No new matter is believed to have been added.

Claims 13-14, 17-24, and 38-39 are rejected under 35 U.S.C. 103(a) over Okada et al., WO00/60112, Miyauchi et al., EP 1148142, and Matsui et al., US 6,194,164.

The rejection is traversed because the combination of the references does not describe or suggest a kit and a method for selective measurement of triglycerides contained in the very low density lipoprotein and intermediate density lipoprotein (as in claims 1 and 13) or very low density lipoprotein (as in claim 40) comprising:

(a) two lipoprotein lipases having different properties, i.e., a first lipoprotein lipase contained in the first reagent that depends on the concentration of a surfactant, and a second lipoprotein lipase contained in the second reagent that hardly depends on the concentration of a surfactant;

(b) two different selective reaction promoters, i.e., two ether or ester compounds of a polyoxyalkylene different in the average added mole number, wherein the m/n ratio is in the range of 1.1 to 1.2, and m is the average mole number of the added polyoxyalkylene ether or ester compound which is used as the first selective reaction promoter and n is the average mole number of the added polyoxyalkylene ether or ester compound which is used as the second selective reaction promoter, and

(c) (a) and (b) provides an advantageous properties to the claimed kit and method.

(a) Two lipoprotein lipases having different properties provides an advantageous properties to the claimed kit and method.

The primary feature of the claimed kit and method is to use two lipoprotein lipases having different in the properties, wherein the activity of the lipoprotein lipase contained in the first reagent depends on the concentration of a surfactant, while that of the lipoprotein lipase contained in the second reagent hardly depends on the concentration of a surfactant.

The effect of this feature is demonstrated by Example 3 of the present specification. See Table 5 on page 54 and "5. Summary" on pages 55-56 of the present specification.

In order to illustrate the results shown in Table 5 of the present specification, the data of Table 5 is presented graphically in Figures 1 to 4 attached with this response. Figures 1 and 2 show the advantageous results provided by the claimed kit and method.

Combination 1 (LP-BP and LPL) of Table 5 on page 54 of the present specification.

The results in Table 5 indicate that when a lipoprotein lipase LP-BP (Asahi Kasei Corporation), which activity depends on the concentration of a surfactant, was used in the first reagent (present in the first step) and a lipoprotein lipase LPL (Asahi Kasei Corporation), which activity hardly depends on the concentration of a surfactant, was used in the second reagent (present in the second step), triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions could be selectively measured, while triglycerides contained in the chylomicron fractions, low density lipoprotein fractions and high density lipoprotein fractions could hardly be measured or be measured only in a very small amount, as illustrated in Figure 1. These results indicate that Combination 1 according to the claimed invention has high sensitivity and selectivity for triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions.

These results also indicate that the degree to which triglycerides contained in the low density lipoprotein fractions were measured was decreased with the increase in the activity level of LP-BP contained in the first reagent (present in the first step). See Figure 1. Combination 2 (LPL-314 and LPL) of Table 5 on page 54 of the present specification.

The results in Table 5 indicate that when a lipoprotein lipase LPL-314 (Toyobo Co., Ltd.), which activity depends on the concentration of a surfactant, was used in the first reagent (present in the first step) and a lipoprotein lipase LPL (Asahi Kasei Corporation), which activity hardly depends on the concentration of a surfactant, was used in the second reagent (present in the second step), triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions could be selectively measured, while triglycerides contained in the chylomicron fractions, low density lipoprotein fractions and high density lipoprotein fractions could hardly be measured or be measured only in a very small amount. See Figure 2. These results indicate that Combination 2 according to the claimed invention has high sensitivity and selectivity for triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions.

These results also indicate that the degree to which triglycerides contained in low density lipoprotein fractions were measured was decreased with the increase in the activity level of LPL-314 contained in the first reagent (present in the first step). See Figure 2. Combination 3 (LPL-BP and LPL-314) of Table 5 on page 54 of the present specification.

The results indicate that when a lipoprotein lipase LP-BP (Asahi Kasei Corporation), which activity depends on the concentration of a surfactant, was used in the first reagent (present in the first step) and a lipoprotein lipase LPL-314 (Toyobo Co., Ltd.), which activity also depends on the concentration of a surfactant, was used in the second reagent (present in the second step), the degree to which triglycerides contained in the very low density

lipoprotein fractions were measured was decreased, compared with that of Combinations 1 and 2 above. However, the degree to which triglycerides contained in the low density lipoprotein fractions were measured was increased, compared with that of Combinations 1 and 2 above. See Figure 3.

These results indicate that Combination 3 has low sensitivity and selectivity for triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions.

Combination 4 (LPL-311 and LPL) of Table 5 on page 54 of the present specification.

These results indicate that when a lipoprotein lipase LPL-311 (Toyobo Co., Ltd.), which activity hardly depends on the concentration of a surfactant, was used in the first reagent (present in the first step) and a lipoprotein lipase LPL (Asahi Kasei Corporation), which activity also hardly depends on the concentration of a surfactant, was used in the second reagent (present in the second step), the degree to which triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions were measured was decreased with the increase in the activity level of LPL-311 contained in the first reagent (present in the first step). See Figure 4.

These results indicate that Combination 4 has low sensitivity and selectivity for triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions.

The concentrations of triglycerides contained in the very low density lipoprotein and intermediate density lipoprotein in test samples differ. During preservation of reagents for measuring triglycerides, the activity of a lipoprotein lipase decreases.

Accordingly, it is impossible to determine exactly triglycerides when the degree to which triglycerides contained in the very low density lipoprotein fractions and intermediate

density lipoprotein fractions are measured is changed with the change in the activity level of a lipoprotein lipase.

However, the degree to which triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions are measured according to the claimed kit and method is hardly changed with the change in the activity level of a lipoprotein lipase. See Figures 1 and 2.

Although the degree to which triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions are measured by using Combination 3 is hardly changed with the change in the activity level of a lipoprotein lipase, Combination 3 has low sensitivity and selectivity for triglycerides contained in the very low density lipoprotein fractions and intermediate density lipoprotein fractions.

Thus, the claimed kit and method provided an advantageous (unexpected) result.

Also, Okada et al., Miyauchi et al., and Matsui et al. do not describe or suggest using two lipoprotein lipases having different properties. Moreover, the cited references are silent with regard to the property of a lipoprotein lipase.

(b) Two different selective reaction promoters, wherein the m/n ratio is in the range of 1.1 to 1.2, provide an advantageous properties to the claimed kit and method.

The claimed kit and method comprise two ether or ester compounds of a polyoxyalkylene different in the average added mole number, wherein the m/n ratio is in the range of 1.1 to 1.2, and m is the average mole number of the added polyoxyalkylene ether or ester compound which is used as the first selective reaction promoter and n is the average mole number of the added polyoxyalkylene ether or ester compound which is used as the second selective reaction promoter.

The effect of the claimed different selective reaction promoters is demonstrated by Example 1 of the present specification. See Table 2 and "5. Summary" on pages 43-46 of the present specification.

The results of Table 2 are illustrated in Figure 5 provided with this response.

The present specification explains the results shown in Table 2 (and in Figure5) as follows:

"5. Summary

5-1: When the second selective reaction promoter is NP-10

The measured results will be described below which were obtained using NP-10 (polyoxyethylene nonylphenyl ether in which the average mole number of the added polyoxyethylene is 10) as the second selective reaction promoter contained in the second reagent (Reagent B) of this invention while varying the average mole number of the added polyoxyethylene in the first selective reaction promoter (polyoxyethylene nonylphenyl ether) contained in the first reagent of this invention (Reagent A).

1'. The results show that when the average mole number of the added polyoxyethylene in the first selective reaction promoter was 10 [NP-10], triglycerides contained in each lipoprotein fraction could not be measured in any one of the lipoprotein fractions: chylomicron fraction, very low density lipoprotein fraction, intermediate density lipoprotein fraction, low density lipoprotein fraction and high density lipoprotein fraction.

2'. The results also show that when the average mole number of the added polyoxyethylene in the first selective reaction promoter was 11 [Emulgen 911], 11.2 [NP-11.2], 11.4 [NP-11.4], 11.5 [NP-11.5] or 11.6 [NP-11.6], triglycerides could be measured in the lipoprotein fractions such as very low density lipoprotein fraction and intermediate density lipoprotein fraction, while they was hardly measured or only a very small amount of them was measured in the lipoprotein fractions such as chylomicron fraction, low density lipoprotein fraction and high density lipoprotein fraction.

This confirms that where the average mole number of the added polyoxyethylene is rounded to the nearest whole number, when the average mole number (n) of the added polyoxyethylene in the second selective reaction promoter is 10 and the average mole number (m) of the added polyoxyethylene in the first selective reaction promoter is 11 to 12 (when the m/n ratio is 1.1 to 1.2), triglycerides contained in very low density lipoprotein and intermediate density lipoprotein can be selectively

measured, compared with those contained in chylomicron, low density lipoprotein and high density lipoprotein.

3'. The results show that when the average mole number of the added polyoxyethylene in the first selective reaction promoter was 13 [NP-13], not only triglycerides contained in the very low density lipoprotein fraction and the intermediate density lipoprotein fraction, but triglycerides contained in the low density lipoprotein fraction were measured.

This confirms that with NP-13, triglycerides contained in very low density lipoprotein and intermediate density lipoprotein or in very low density lipoprotein cannot be selectively measured.

5-2: When the first selective reaction promoter is Emulgen 911

The measured results will be described below which were obtained using Emulgen 911 (polyoxyethylene nonylphenyl ether in which the average mole number of the added polyoxyethylene is 11) as the first selective reaction promoter contained in the first reagent (Reagent A) of this invention while varying the average mole number of the added polyoxyethylene in the second selective reaction promoter (polyoxyethylene nonylphenyl ether) contained in the second reagent of this invention (Reagent B).

1'. The results show that when the average mole number of the added polyoxyethylene in the second selective reaction promoter was 10 [NP-10], triglycerides contained in the very low density lipoprotein fraction and the intermediate density lipoprotein fraction could be measured, while triglycerides contained in the chylomicron fraction, the low density lipoprotein fraction and the high density lipoprotein fraction could hardly be measured.

The experimental results also confirms that when the average mole number (m) of the added polyoxyethylene in the first selective reaction promoter is 11 and the average mole number (n) of the added polyoxyethylene in the second selective reaction promoter is 10 (when the m/n ratio is 1.1), triglycerides contained in very low density lipoprotein and intermediate density lipoprotein can be selectively measured, compared with those contained in chylomicron, low density lipoprotein and high density lipoprotein.

2'. The results also show that when the average mole number of the added polyoxyethylene in the second selective reaction promoter was 11.2 [NP-11.2], 11.4 [NP-11.4], 11.6 [NP-11.6] or 13 [NP-13], triglycerides could be measured in the lipoprotein fractions such as very low density lipoprotein fraction and intermediate density lipoprotein fraction; however, the percentage was low.

> This confirms that where the average mole number of the added polyoxyethylene is rounded to the nearest whole number, the measurement reagents in which the average mole number (m) of the added polyoxyethylene in the first selective reaction promoter is 11 and the average mole number (n) of the added polyoxyethylene in the second selective reaction promoter is 11 to 13 (when the m/n ratio is 0.85 to 1.0) are not suitably used for the selective measurement of triglycerides contained in very low density lipoprotein and intermediate density lipoprotein or in very low density lipoprotein."
>
> Pages 43-46 of the present specification.

Thus, as describe in Table 2 and pages 43-46, the claimed kit and method provide an advantageous (unexpected) result.

The claimed kit and method.

The claimed kit and method allow an easy and accurate selective measurement of triglycerides ("TG") contained in VLDL and IDL (page 3 of the present specification). Since the triglycerides contained in the low density lipoprotein and the high density lipoprotein have been already eliminated in the first step, only the triglycerides contained in the very low density lipoprotein and intermediate density lipoprotein or in the very low density lipoprotein react with lipoprotein lipase to generate glycerol in the second step (see pages 8-10). When adding catalase to the first reagent as an enzyme that catalyzes the reaction leading to the conversion of hydrogen peroxide unto another substance, a substance that inhibits the activity of the catalyse (e.g., sodium azide) is added to the second reagent so that the hydrogen peroxide generated in the second step is not eliminated by the catalyse. However, when adding peroxidase to the first reagent as an enzyme that catalyzes the reaction leading to the conversion of hydrogen peroxide into another substance, a substance that inhibits the activity of the enzyme is not added to the first reagent (see page 35, lines 4-11, and pages 20-22).

Disclosure of the cited references.

Okada et al. describe reagents for measuring triglycerides in a sample comprising two reagents. A first reagent comprises glycerol kinase, glycerol-3-phosphate oxidase, and

catalase, while a second reagent comprises a lipoprotein lipase (see page 35, working example 1 of the automated English translation).

Miyauchi et al. describe a method of quantifying TG in HDL and LDL (see [0025]-[0030] and [0038]-[0048] and the Examples) using only one lipoprotein lipase. For measuring TG in HDL, reagents for inhibiting the reaction of lipoproteins other than HDL (e.g., aggregating agents) are added to prevent decomposition of TG in LDL and VLDL by a lipoprotein lipase and then the free glycerol contained in the sample is eliminated ([0026]-[0029]). Subsequently, a lipoprotein lipase and other enzymes are added to generate hydrogen peroxide from HDL [0029]. A similar process is used for quantifying TG in LDL.

Matsui et al. describe a reagent for measuring cholesterol in LDL comprising the cholesterol esterase and cholesterol oxidase in the first reagent (col. 2, lines 59-62; tables 1-2 and 4). Matsui et al. do not describe using a lipoprotein lipase.

Further, Matsui et al. describe using specific surfactants having specific different hydrophilic-lipophilic balance ("HLB") in the first and second measurement steps, e.g., HLB is 13-15 in the first step, and 11-13 in the second step (see col. 3-4).

Matsui et al. do not teach adjusting HLB values so as surfactants act on different lipoproteins, e.g., IDL and VLDL, and different lipoprotein lipases, not to mention, adjusting a ratio of the average amount of moles of polyoxyalkylene in its ether or ester compound.

The HLB value reflects a degree to which a surfactant is hydrophilic or lipophilic (see the enclosed pages from "Wikipedia", accessed at http://en.wikipedia.org on 12/5/2007, 2 pages, submitted previously). The HBL value does not show how many moles of a surfactant is added and a ratio of the surfactants in two steps.

Thus, Okada et al., Miyauchi et al., and Matsui et al. do not describe or suggest using two different lipases having different activities in two different reagents and selecting a specific molar ration of the two selective reagents.

As described in the present specification, the claimed kit provides high selectivity to VLDL and IDL (e.g., pages 43-46). When the average mole number ration is 1.1.-1.2, the triglycerides content in VLDL and IDL can be selectively measured, compared to those contained in chylomicron, LDL, and HDL (page 44, third full paragraph).

The Examiner is of the opinion that it would have been obvious to modify the reagent of Okada et al. and select a particular mole number ratio of the polyoxyalkylene derivatives based upon the teaching of the calculation of HLB values and art-recognized method of using surfactants with the particular HLB values to stabilize specific lipoproteins.

However, one would not have been motivated to modify Okada et al. with the disclosures of Miyauchi et al. and Matsui et al. because the methods and reagents of Okada et al., Miyauchi et al., and Matsui et al. are different from each other, they use different enzymes, measure different substance (triglycerides or cholesterol), and exhibit different behaviors towards surfactants such as polyalkylene oxide derivatives.

The Examiner has taken a position that Applicants' arguments with regard to the combination of Okada et al., Miyauchi et al., and Matsui et al. are not persuasive because the test is "what the combined teachings of the references would have suggested to those of ordinary skilled in the art." Page 10, last paragraph of the Official Action.

In response, it is noted that since the disclosures of Okada et al. with respect to the selecting HLB contradicts to the disclosure of Matsui et al., one would not have known how to modify HLB values and what surfactants and HLB values to select for measuring triglycerides contained in various lipoproteins based on the "*combined teachings*" of Okada et al. and Matsui et al. One would not have reasonably expected to achieve the claimed kit and method based on the contradictory discloses of the cited references and would have to conduct actual experiments to find out what modifications are acceptable based on the contradictory "*combined teachings*" of Okada et al. and Matsui et al.

More specifically, Okada et al. describes that BL-9EX (POE(9) lauryl ether) which is a polyalkylene oxide derivative can make an enzyme such as lipoprotein lipase selectively react with triglycerides in LDL and HDL (see page 39, eighth paragraph, of the automated English translation). BL-9EX has HLB value 14.5 (see Annex 1). Matsui et al. describe that polyalkylene oxide derivatives having HLB values of not less than 13 and not more than 15 can make cholesterol esterase and cholesterol oxidase selectively react with cholesterol in lipoproteins other than LDL (i.e., HDL, VLDL, CM and the like; col. 3, lines 17-37).

Further, Okada et al. describe that KF-351 (polyether-modified silicone oil) which is polyalkylene oxide derivative can make an enzyme such as lipoprotein lipase selectively react with triglycerides in IDL and HDL (see page 39, seventh paragraph, of the automated English translation). KF-351 has HLB value of 12 (see Annex 2 and 3). Matsui et al. describe that polyalkylene oxide derivatives having HLB values of not less than 11 and not more than 13 can make a cholesterol esterase and cholesterol oxidase selectively react with cholesterol in all lipoproteins (i.e., HDL, VLDL, CM, and LDL; col. 4, lines 12-39).

Thus, it would not have been obvious what surfactants and HLB values to select based on the contradictory data of Okada et al. and Matsui et al. for the selective measurement of TG in VLDL and IDL.

Thus, Okada et al., Miyauchi et al., and Matsui do not make the claimed kit and method obvious.

Applicants request that the rejection be withdrawn.

A Notice of Allowance for all pending claims is requested.

Respectfully submitted,

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FIG. 1

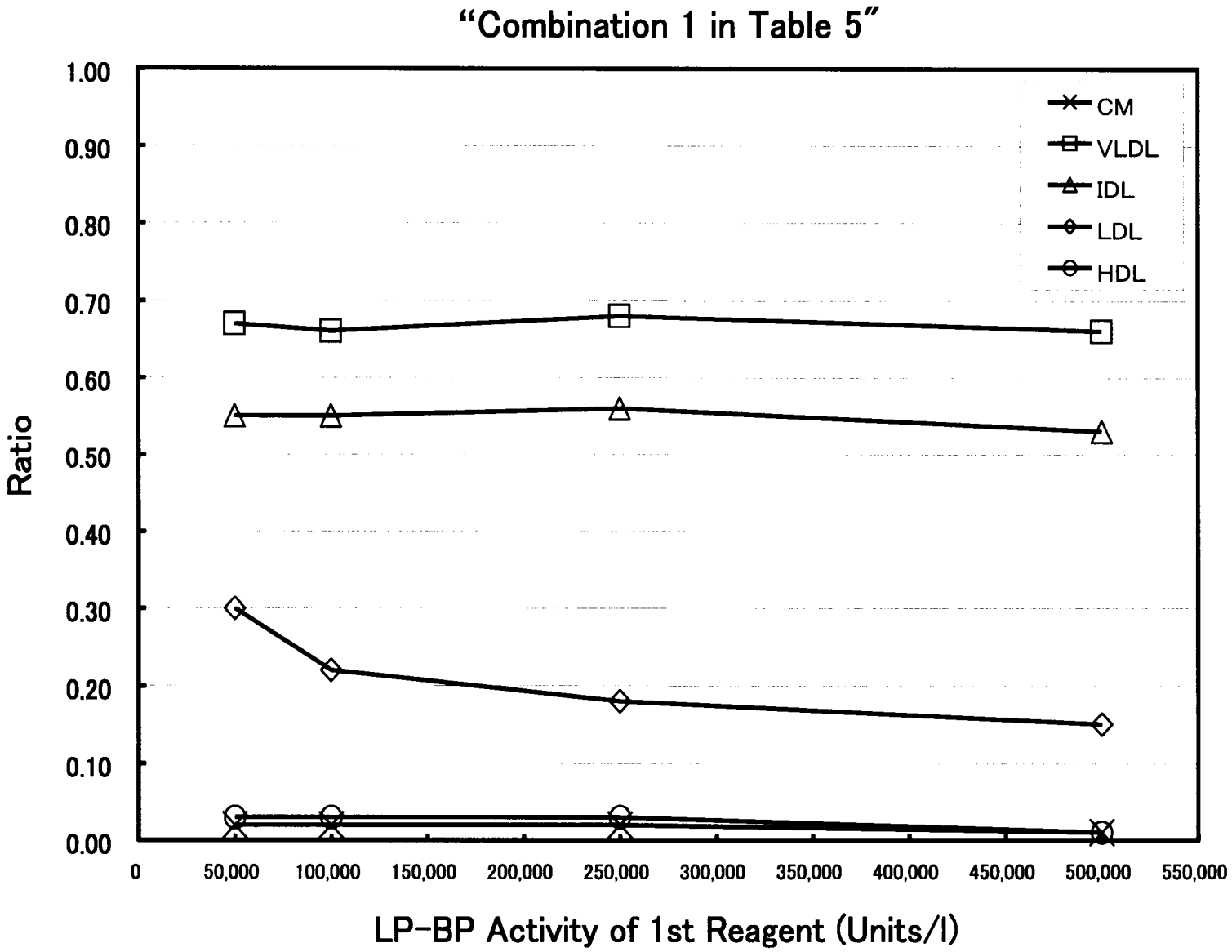

"Combination 1 in Table 5"
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Ratio
CM
VLDL
IDL
LDL
HDL
0
50,000
100,000
150,000
200,000
250,000
300,000
350,000
400,000
450,000
500,000
550,000
LP-BP Activity of 1st Reagent (Units/l)

FIG. 2

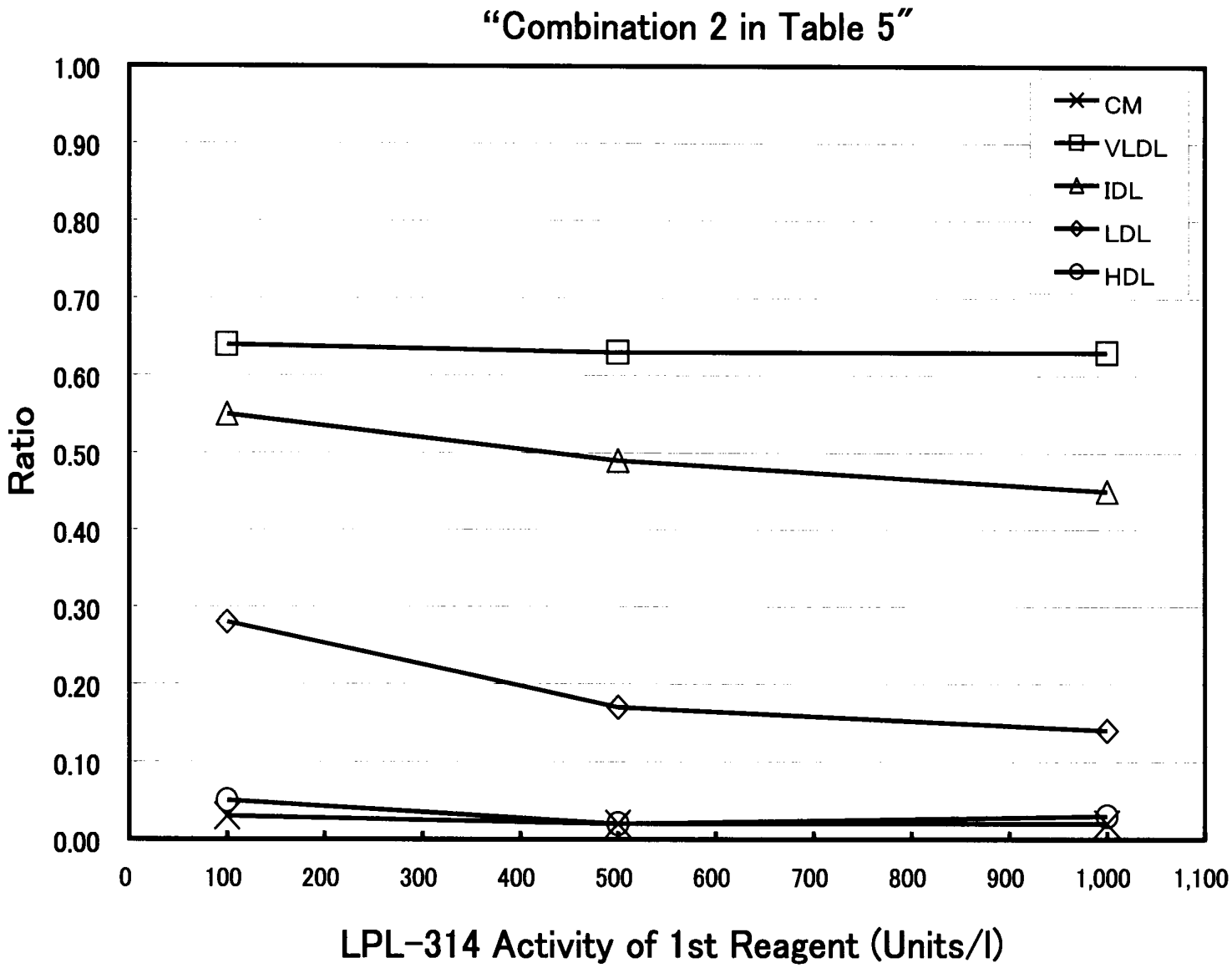

"Combination 2 in Table 5"
Ratio
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
100
200
300
400
500
600
700
800
900
1,000
1,100
LPL-314 Activity of 1st Reagent (Units/l)
CM
VLDL
IDL
LDL
HDL

FIG. 3

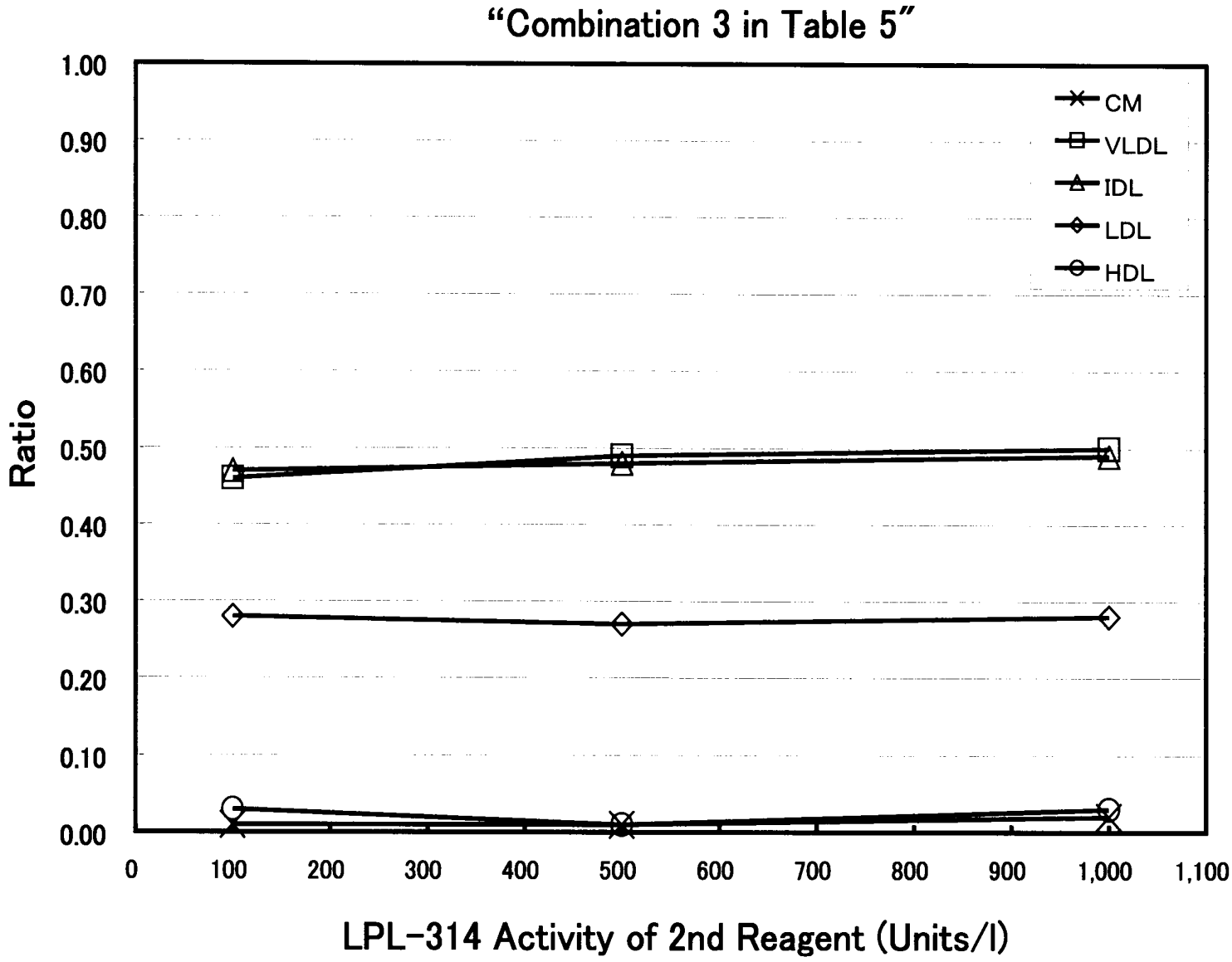
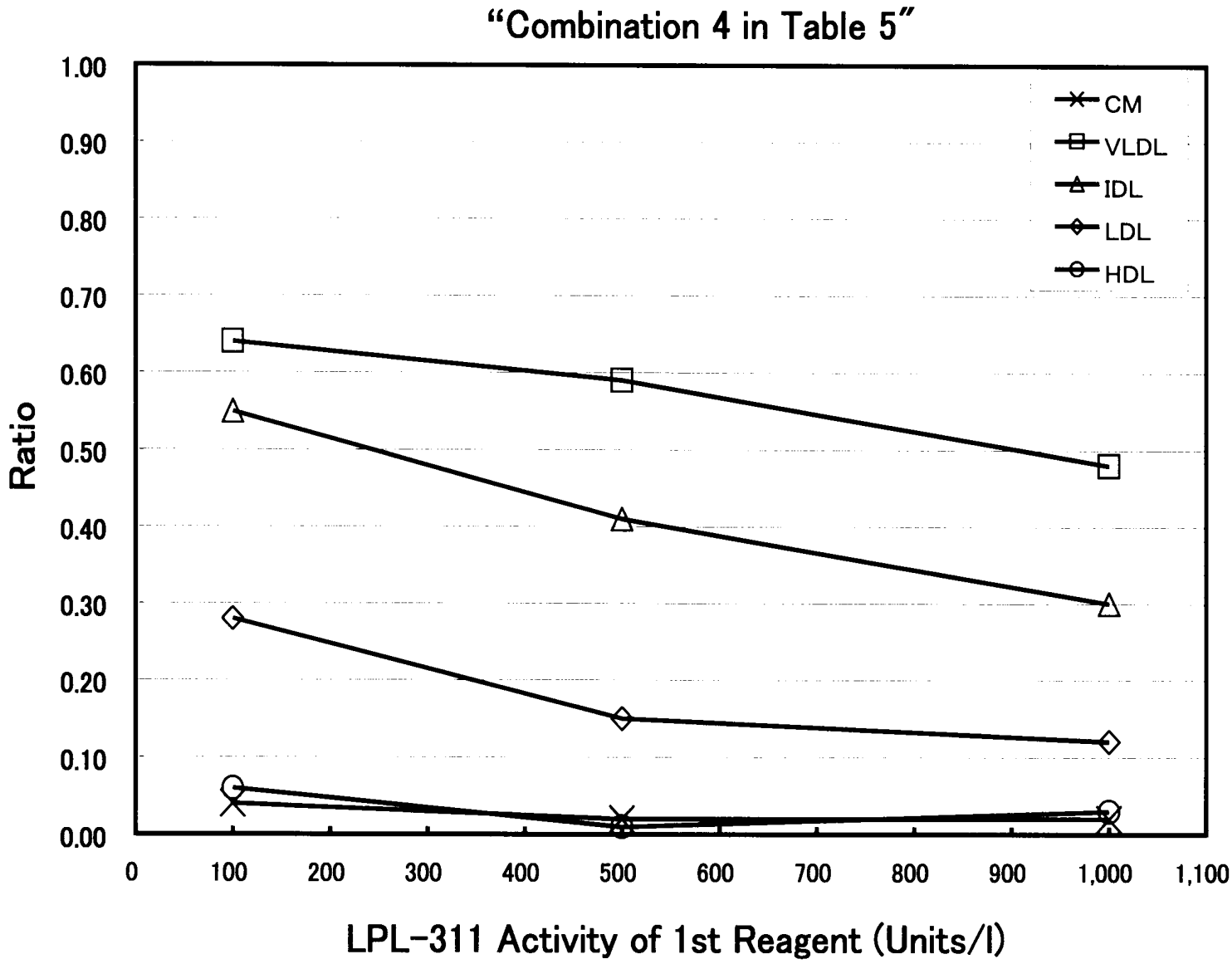

"Combination 3 in Table 5"
Ratio
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
100
200
300
400
500
600
700
800
900
1,000
1,100
CM
VLDL
IDL
LDL
HDL
LPL-314 Activity of 2nd Reagent (Units/l)

FIG. 4


"Combination 4 in Table 5"
Ratio
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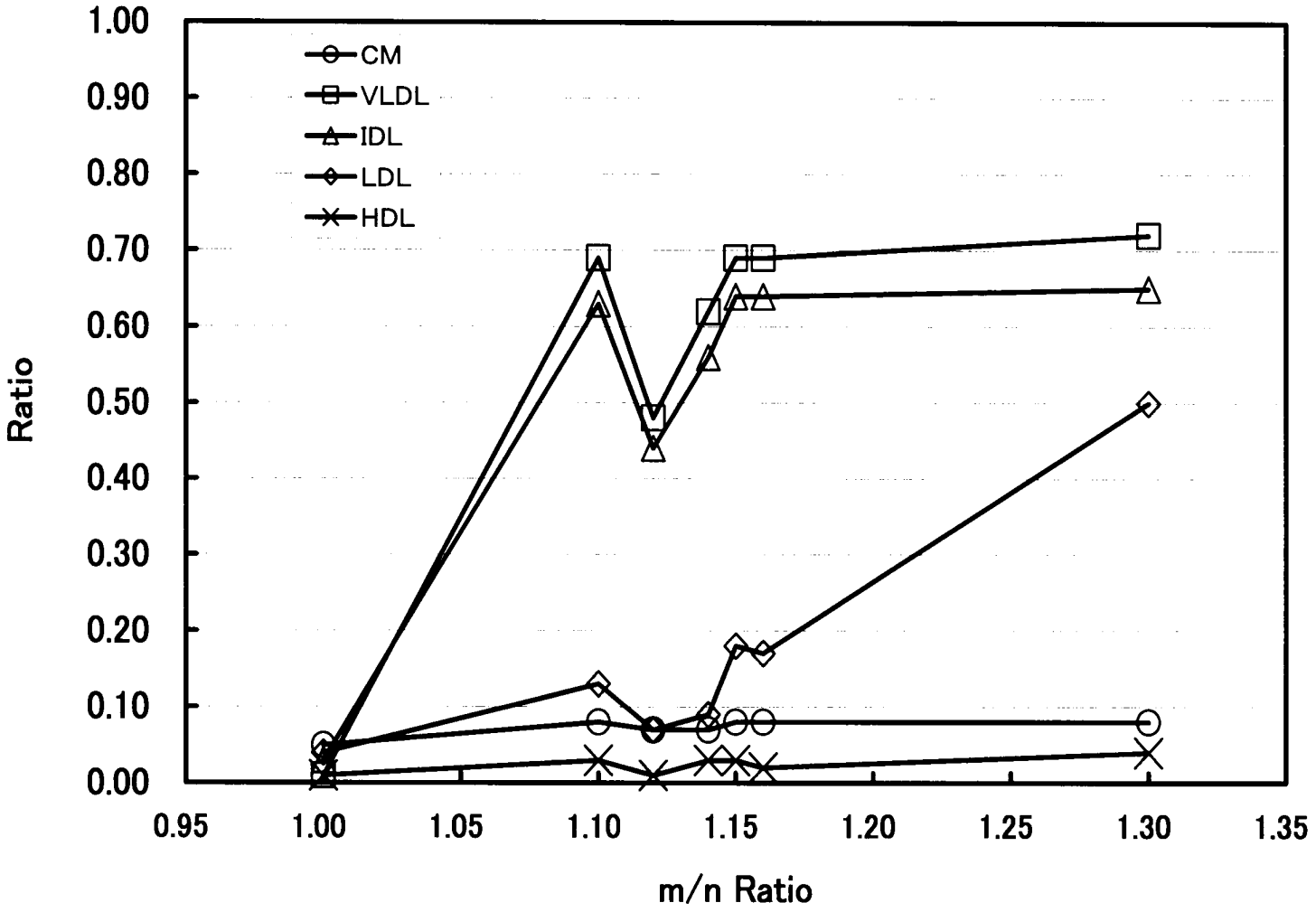
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
100
200
300
400
500
600
700
800
900
1,000
1,100
LPL-311 Activity of 1st Reagent (Units/l)
CM
VLDL
IDL
LDL
HDL

FIG. 5


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Ratio
CM
VLDL
IDL
LDL
HDL
0.95
1.00
1.05
1.10
1.15
1.20
1.25
1.30
1.35
m/n Ratio